C2 cont.

25. (Amended) The method according to claim 19, wherein the adjuvant and the antigen are administered subcutaneously, transcutaneously or intramuscularly.

II. REMARKS

Claims 1-30 are presently pending in this application. Claims 1-18 have been withdrawn pursuant to a restriction requirement. Claims 19-30 stand variously rejected under 35 U.S.C. §§ 112, 102 and 103.

By amendment herein, claims 19 and 21-25 have been amended. Claim 20 has been canceled, without prejudice or disclaimer. Support for the amendments can be found throughout the specification and claims as originally filed. For instance, claim 19 has been amended to incorporate the provisions of claim 20 and claims 21 and 25 have been amended to correct typographical errors. Claim 22 has been amended to eliminate the Markush group language and claims 23 and 24 have been amended to properly depend from claim 19 rather than canceled claim 20. Thus, no new matter has been added to the application by way of the amendment and entry of these amendments is respectfully requested.

Overview of the Invention

The pending claims are drawn to methods of immunizing a vertebrate subject by administering an adjuvant comprising a detoxified mutant of a cholera toxin (CT) or an *E. coil* heat labile toxin in combination with at least one antigen. One key requirement of these methods is that the adjuvant and antigen are administered parenterally. In other words, as defined by Applicants on page 7, lines 21-26 of the specification, parenteral administration refers to "introduction into the body outside of the digestive tract, such as by subcutaneous, intramuscular, transcutaneous, intradermal, or intravenous administration. This is to be contrasted with adjuvants that are delivered to a mucosal

surface, such as oral, intranasal, vaginal, or rectal." Thus, the present invention provides methods using detoxified CT or LT toxins as parenteral adjuvants.

Drawings

Applicants acknowledge receipt of the Draftsperson's Form PTO 948. Upon indication of allowed claims, Applicants will submit corrected drawings that comply with 37 C.F.R. 1.84.

Oath/Declaration

The Office Action mistakenly indicates that the oath/declaration is defective. However, upon further communications, Examiner Devi indicated that this objection had been issued before a signed declaration was matched with the file. Accordingly, in a telephone message left on September 13, 1999, Examiner Devi instructed us to disregard this objection and indicated that the oath was not defective.

Rejections Under 35 U.S.C. § 112, First Paragraph

Claim 22 stands rejected under 35 U.S.C. § 112, first paragraph as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to use the claimed invention. In particular, it is alleged that it would require undue experimentation to practice the invention using detoxified mutant proteins CT-S109 or LT-R72.

Applicants traverse this rejection.

It is axiomatic that an applicant's claims are not limited in scope to those embodiments actually disclosed or exemplified in the specification. see, e.g., *Spectra-Physics Inc. v. Coherent Inc.,* 3 USPQ2d 1737 (Fed. Cir. 1987). Furthermore, without reason to doubt the truth of the statements made in the patent application, the application must be considered enabling. see, *In re Wright*, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993)

and *In re Marzocchi*, 169 USPQ 367, 369 (CCPA 1971). Thus, the burden is on the Office to establish why the claimed invention is not enabled by the specification.

The Examiner in the pending case has not satisfied this burden. The Examiner maintains that two mutant species of original claim 22, CT-S109 and LT-R72, are not enabled by Applicants' specification and cites Pizza et al. (Molec. Microb.) as alleged providing evidence that LT-R72 and CT-S109 are not a "detoxified" mutants. However, Applicants direct the Examiner's attention to Example 5, on page 33 of their specification. In this Example, immunization of mice with LT-R72 is described and demonstrated. Mice used in this experiment survived up to 80 days, indicating, in direct contrast to Pizza et al., that this particular mutant is indeed detoxified. Applicants also incorporate by reference the disclosure of Giuliani et al. (page 33, line 23), which describes how to make the LT-R72 mutant. Accordingly, the specification enables the claimed LT-R72 mutant.

Furthermore, submitted herewith is copy of WO 98/18928 (Exhibit A), published May 7, 1998, which demonstrates that LT-R72 is indeed detoxified. In particular, Figures 4 and 5 show *in vitro* and *in vivo* toxicity experiments, respectively, and show that LT-R72 is non-toxic.

With respect to CT-S109, Pizza et al. is silent as to any substitutions at position 109 of CT and, accordingly, is not evidence that this mutant is toxic. Moreover, Applicants' specification teaches all that is necessary to make and use the claimed mutants in the claimed methods. In particular, methods of making the appropriate amino acid substitution (*e.g.*, page 14, line 23 to page 15, line 30 and Examples) and methods of testing mutants for toxicity (*e.g.*, page 7, lines 27 to page 8, line 8) are described. In addition, methods of immunizing and evaluating vertebrate subjects are similarly detailed (*e.g.*, page 9, lines 9-30 and Examples) in the specification. Thus, the CT-S109 mutant is also adequately enabled.

Further evidence establishing that the claimed CT-S109 mutant is non-toxic, both *in vitro* in Y1 cells and *in vivo* in rabbit ileal loop, is provided in Exhibit B. This manuscript and accompanying Figures establishes that CT-S109 is non-toxic. (see, Figure 1 and pages 12-13).

In sum, the rejection under section 112, first paragraph is improper and Applicants respectfully request the rejection be withdrawn.

35 U.S.C. § 112, Second Paragraph

Claims 21-24 stand rejected under 35 U.S.C. § 112, second paragraph as allegedly indefinite for certain typographical errors and for alleged lack of antecedent basis.

These claims have been amended to obviate these rejections. The amendments are made without conceding the correctness of the Examiner's position and are made solely to advance prosecution. In view of the foregoing amendments and remarks, the rejections under section 112, second paragraph have all been addressed and Applicants respectfully request these rejections be withdrawn.

Rejections Under 35 U.S.C. § 102

The claims stand rejected as allegedly unpatentable under section 102 based on five references. Applicants address each rejection in turn.

Rejections based on Tommaso, Douce and Rappuoli

Claims 19-23 and 30 are rejected under 102(b) as allegedly anticipated by Tommaso et al. (Infect. Immun. 64:974-979, 27 February 1996). Claims 19-21, 25, 26 and 30 are rejected under 102(b) as allegedly anticipated by Douce et al. (PNAS 92:1644-1648, February 1995). Claims 19-21, and 25-30 are rejected under 102(b) as allegedly anticipated by Rappuoli et al. (WO 95/17211, published 06/29/95).

Tommaso is cited for teaching a method of immunizing mice by intravaginal administration of an antigen and a detoxified mutant, namely LTK63. Douce and Rappuoli are cited for teaching use of LTK7 as a mucosal adjuvant.

Tommaso, Douce and Rappuoli each fail to describe and demonstrate elements of the pending claims. First, the references do not describe parenteral administration, as required in Applicants' methods. In particular, these three references describe only mucosal immunization regimes. The Office acknowledges that Tommaso is limited to intravaginal administration methods. (Office Action, page 6). Applicants note that Douce and Rappuoli are similarly limited (see, *e.g.*, Titles and Abstracts).

Second, Douce and Rappuoli fail to teach or suggest methods in which the non-toxic mutant is an adjuvant. Rather, with regard to subcutaneous (s/c) immunization methods, these references teach that s/c administration of an antigen alone (*e.g.*, Ova) or antigen with toxin (mutant or native) produce similar immune responses. (see, Figure 2 in Douce and Figure 1 in Rappuoli). Thus, Douce and Rappuoli fail to teach that parenterally-administered detoxified CT, LT and PT mutants act as adjuvants. Since Tommaso, Douce and Rappuoli all fail to teach or suggest elements of the pending claims, none of the pending claims are anticipated by these references. Accordingly, withdrawal of these rejections is respectfully requested.

Rejections Based on Gajewzyk

Claims 19-22, 25, 26, and 28-30 are rejected under 102(b) as allegedly anticipated by Gajewzyk et al. (WO 95/34323, published 12/21/95). Gajewzyk is cited for teaching administration of a non-toxic adjuvant comprising a detoxified mutant of a *Bordetella pertussis* ADP-ribosylating toxin, K9G129.

Applicants traverse. The pending claims are limited to methods which involve the use of a detoxified CT or LT mutant. Thus, Gajewzyk discussion of PT mutants is not

applicable to the claims. Accordingly, withdrawal of this rejection is respectfully requested.

Rejections Based on Pizza

Claims 19-23, and 25-30 are rejected under 102(a) as allegedly anticipated by Pizza et al. (WO 97/02348, published 01/23/95). Pizza is cited as allegedly disclosing a method of immunizing a vertebrate subject by administering an effective amount or dose of a composition comprising a detoxified mutant protein of a bacterial toxin.

Applicants traverse. It is well-settled that in order to constitute an anticipatory reference, the cited document must contain an enabling disclosure. *Chester v. Miller*, 15 USPQ2d 1333, 1336 n.2 (Fed. Cir. 1990); see also, *Scripps Clinic & Research Foundation v. Genentech, Inc.,* 18 USPQ2d 1001, 1011 (Fed. Cir. 1991). In other words, the reference must teach one of skill in the art how to practice the claimed invention, without undue experimentation.

In the case at hand, Pizza's disclosure does not enable the claimed methods. As noted above, the claimed methods are specifically directed to methods of parenteral administration and methods in which the detoxified LT or CT mutant acts as an adjuvant. Pizza contains no teaching or guidance in either of these regards. Rather, the reference is directed to methods which use certain detoxified CT or LT mutants as antigens. The two statements in Pizza (page 15 and page 16) which are relied on by the Office as allegedly suggesting use of the mutants as adjuvants, are unsubstantiated, general statements and do not provide the guidance necessary to lead one of skill in the art to the claimed invention. Thus, because Pizza does not teach, suggest or enable the claimed methods, none of the pending claims are anticipated by this reference and withdrawal of this rejection is respectfully requested.

Rejections Under 35 U.S.C.§ 103(a)

Claims 22-24 stand rejected as allegedly obvious over Rappuoli et al. (WO 95/17211 published 06/29/95) as applied to claims 19-21 above, and further in view of Roberts et al. (Infect. Immun. 63:2100-2108, June 1995), or Partidos et al. (Immunology 89:483-487, December 1996).

Rappuoli is cited as above for allegedly teaching the use of LTK7 as a mucosal adjuvant. Roberts et al. is alleged to teach a detoxified PT mutant and methods of immunizing using this mutant. Partidos is cited for teaching that a detoxified LT mutant produces LT-specific antibodies. It is maintained that

> "it would have been *prima facie* obvious to one of skill in the art at the time the invention was made to use Roberts' detoxified mutant of a ADP-ribosylating pertussis toxin, PT-9K/129, or Partidos's or Pizza's detoxified mutant of a ADP-ribosylating *E. coli* heat-labile toxin, LT-K63, as alternative adjuvant having similar functions, in place of Rappuoli's LTK7 mutant in Rappuoli's method of immunizing mice by parenteral administration, to produce the instant invention for the expected benefit of also eliciting LT- or PT-specific protective antibodies as taught by Partidos et al. or Roberts et al., in addition to enhancing the antibody response to the selected second antigen." (Office Action, page 9).

Applicants traverse this rejection. For the reasons detailed above, Applicants reiterate that Rappuoli does not teach or suggest the claimed methods. Specifically, Rappuoli does not teach parenteral administration of detoxified mutants and, furthermore, does not describe or demonstrate that these mutants are useful as adjuvants.

The secondary references fail to supply what is missing from Rappuoli. Neither Roberts nor Partidos describe or suggest that a subject could be immunized against a selected antigen by parenterally administering a detoxified LT or CT mutant in combination with the selected antigen. Thus, combining Rappuoli with Roberts, Partidos or Pizza would not lead one of skill in the art to the claimed invention. Accordingly, the 103 rejection is improper and Applicants respectfully request that it be withdrawn.

III. CONCLUSION

In view of the foregoing, Applicants submit that the claims are now in condition for allowance and requests early notification to that effect.

Please direct all further communications regarding this application to:

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